Nitrate fertilization increases *Kiwifruit plant* tolerance to *Pseudomonas syringae* pv. *actinidiae*



M. Nunes da Silva^{1,2}, A. P. G. Fernandes¹, M. W. Vasconcelos^{2*} and S. M. P. Carvalho^{1*}

¹ GreenUPorto – Research Centre on Sustainable Agrifood Production, Faculty of Sciences of University of Porto, Vairão, Portugal. ²Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal.

*E-mails: mvasconcelos@porto.ucp.pt; susana.carvalho@fc.up.pt



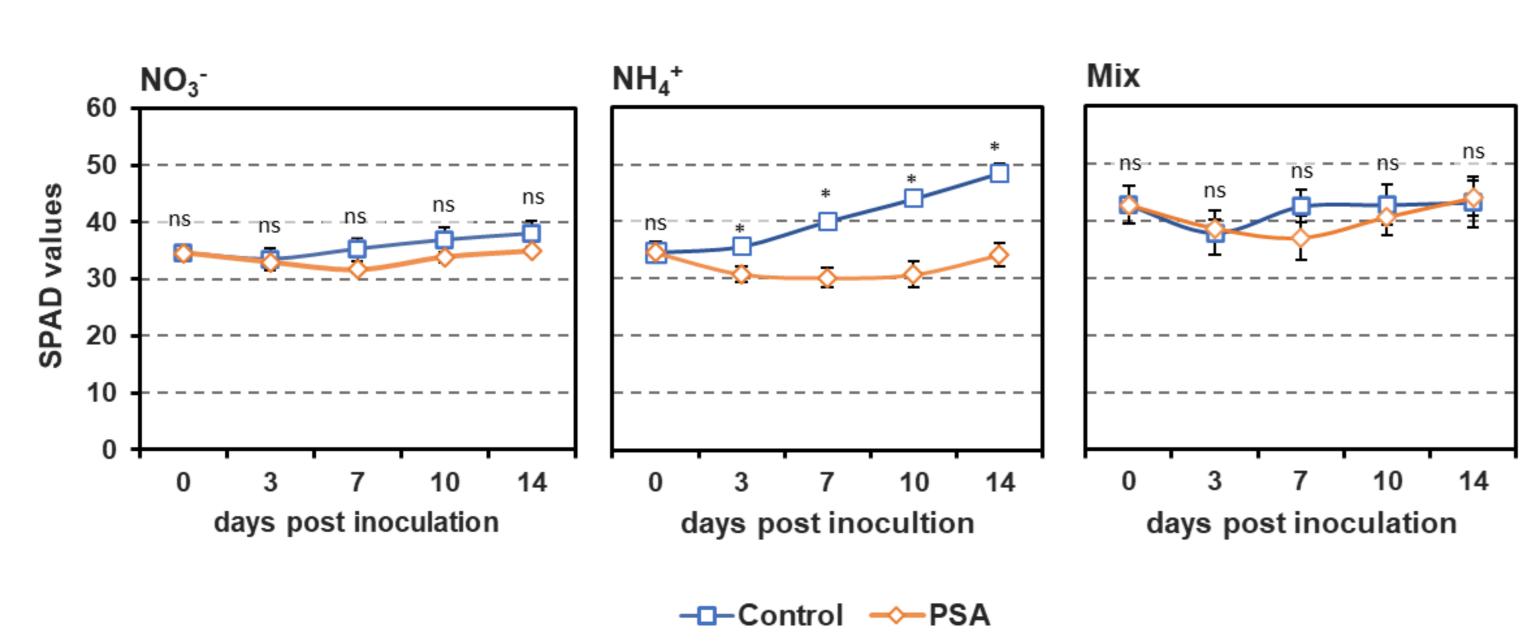
Introduction

Pseudomonas syringae pv. actinidiae (Psa) is the pathogen responsible for the kiwifruit bacterial canker (KBC), for which no curative methods have been developed yet. The source of nitrogen (N) may have consequences on overall plant nutritional status, which in turn may also affect the plant's predisposition for pathogen infection. However, the lack of knowledge on how kiwifruit plants (Actinidia spp.) respond to infection by Psa when grown under different N supplies hinders the possibilities to use N supply in integrated approaches to diminish disease severity. The aim of this study was to understand how nitrate (NO₃-) and ammonia (NH₄+) modulate plant defence mechanisms against Psa, paving the way for the development of novel N fertilization regimens that increase plant resilience to the pathogen, or that ensure plant growth and productivity even with Psa infection.

Methods

- 1. A. chinensis var. deliciosa cv. 'Hayward' where grown for 21 days in a hydroponics system with nutritive solutions differing in the type of N supply: 214 μ M NO₃⁻, 214 μ M NH₄⁺ or a mixture of both (Mix 107 μ M NO₃⁻ + 107 μ M ppm NH₄⁺).
- 2. Psa was inoculated onto plant leaves by rubbing the abaxial surface with an infected swab.
- 3. Fourteen days post inoculation (dpi) plants were sampled for the analysis of: photosynthetic capacity, Psa endophytic population, total N, mineral composition and gene expression.

Results and Discussion



Photosynthetic capacity of plants under NH₄⁺ supply decreased along the experimental trial.

Fig. 1 – Photosynthetic capacity measured as SPAD values.

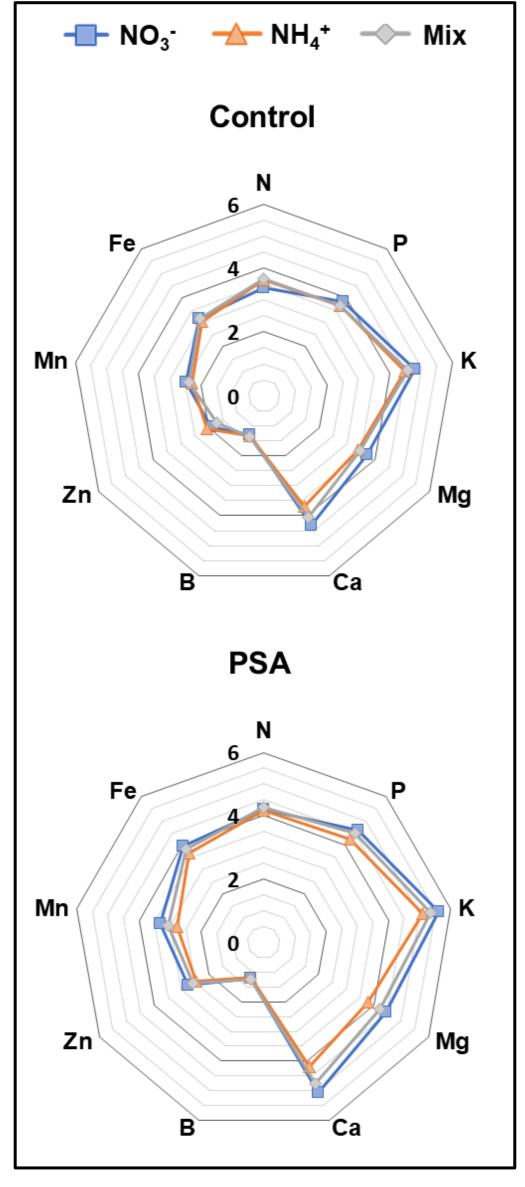
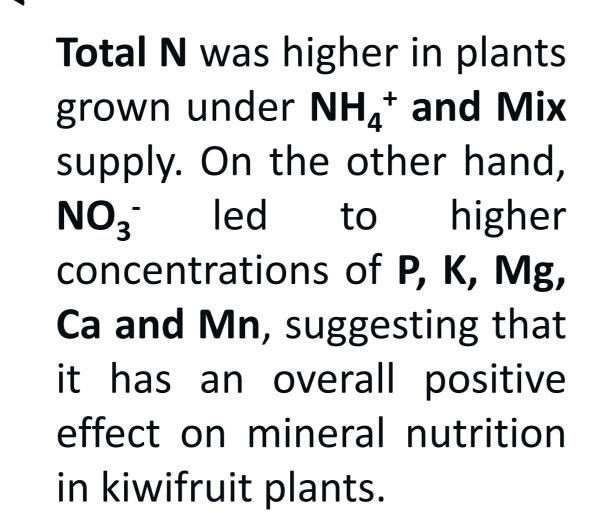


Fig. 3 – Mineral accumulation in plant tissues (mg in log values).

Supplementation with NO₃- led to lower Psa endophytic population in plant tissues, whereas NH₄+ and Mix led to higher infection rate.



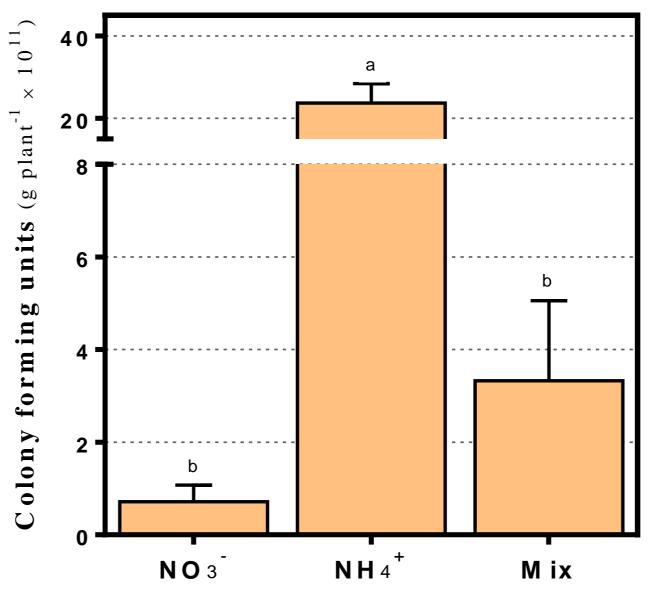


Fig. 2 – Density of Psa population in plant tissues.

NH₄⁺ induced the expression of genes related to **plant stress** (*PR1*) and **secondary metabolism** (*LOX, PAL* and *SAM*). Genes related with **N metabolism** were differently regulated **depending on the N source**: whereas *GLU1* and *GDH1* were overexpressed with NH₄⁺ and Mix, NO₃⁻ led to overexpression of *GAD1*.

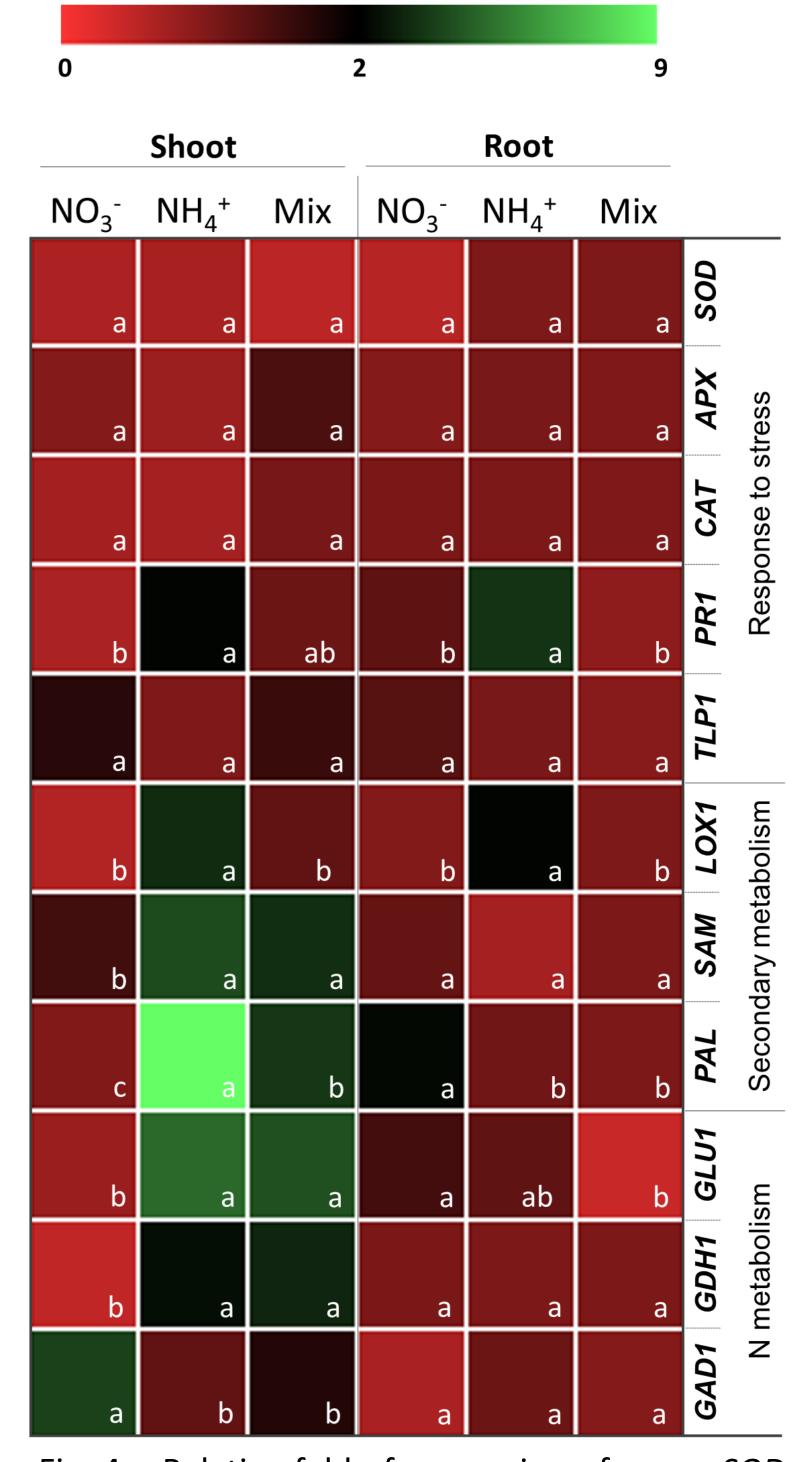


Fig. 4 – Relative fold of expression of genes *SOD* (superoxide dismutase), APX(ascorbate peroxidase), CAT (catalase), PR1 (pathogenesisrelated protein 1), TLP1 (thaumatin-like protein 1), LOX1 (lipoxygenase 1), PAL (phenylalanine ammonia lyase), SAMs (s-adenosylmethionine synthetase 1), GLU1 (glutamate synthase 1) and GDH1 (glutamate dehydrogenase) and GAD1 (glutamate decarboxylase). Within each structure (shoot or root), different letters represent statistically different means at p < p0.05. In red: no alteration or downregulation of gene expression; in black/green: upregulation of gene expression.

Conclusions

- ✓ NO₃ led to lower Psa colonization and maintenance of plant photosynthetic capacity, having potential to be included in integrated pest management strategies against Psa.
- \checkmark NO₃⁻ could have increased plant tolerance to the pathogen by improving P, K, Mg, Ca and Mn nutrition.
- ✓ The higher levels of total N in NH₄⁺ and Mix-supplied plants may have underpinned the increased Psa colonization observed with these treatments.







